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Role of the NF-κB signaling pathway in the pathogenesis of colorectal cancer

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Abstract

The NF-κB signaling pathway is a key regulator of CRC cell proliferation, apoptosis, angiogenesis, inflammation, metastasis, and drug resistance. Over-activation of the NF-κB pathway is a feature of colorectal cancer (CRC). While new combinatorial treatments have improved overall patient outcome; quality of life, cost of care, and patient survival rate have seen little improvement. Suppression of the NF-κB signaling pathway using biological or specific pharmacological inhibitors is a potential therapeutic approach in the treatment of colon cancer. This review summarizes the regulatory role of NF-κB signaling pathway in the pathogenesis of CRC for a better understanding and hence a better management of the disease.

Key words: NF-κB signaling, Tumorigenesis, Pharmacological inhibitors, Colorectal cancer

Introduction

Colorectal cancer (CRC) is the fourth most common cause of cancer-related mortality, with approximately 700,000 patients dying globally per annum (1, 2). Patients with CRC are more prevalent in developed countries with westernized lifestyles and high alcohol consumption. Aging, high body mass index (BMI), positive smoking habit, extant polyposis, and inflammatory bowel disease (IBD) enhance the risk of colorectal cancer incidence (2-6). Colorectal cancer is a heterogeneous disease with the majority of cases being sporadic (at least 80% of CRC patients) (7). However, there is a minor opulation that is categorized as an inherited group with specific genetic mutations. Patients with hereditary non-polyposis colorectal cancer (HNPCC), familial adenomatous polyposis (FAP), or the polyposis syndromes comprise approximately 5% of CRC population (8). This heterogeneity is responsible for different clinical outcomes, patient survival and therapeutic responses in colorectal cancer (9). Screening for CRC is important to decrease its occurrence and mortality (6). The combination of antibody and chemotherapeutic agents or other combination therapies against metastatic CRC have given rise to a significant reduction in the malignant features of the disease and increased survival (10-13).

Nuclear Factor-kappa B (NF-κB) is a ubiquitous transcription factor that mediates a cytoplasmic/nuclear signaling pathway (14) and regulates gene expression of various cytokines, cytokine receptors and adhesion molecules involved in inflammatory and immune reactions (15). Furthermore, there is a correlation between the activation of NF-κB and control of apoptotic pathway, cell proliferation, differentiation, migration, and angiogenesis as well as resistance to chemo/radiotherapies in tumor cells (16). The important role of NF-κB is recognized in several cancers including breast cancer (17), ovarian cancer (18), prostate cancer (19), gastric carcinoma (20), and colorectal cancer (21). Targeting NF-κB, may lead to preventive measures and novel treatment approaches against human tumors (22). In this review, we summarize the therapeutic potency of NF-κB pharmacological inhibitors against

colorectal cancer initiation/progression as a novel therapeutic approach for better management of CRC.

Nuclear Factor-kappa B signaling pathways in colorectal cancer

NF-κB is a heterodimer protein, that consists of two subunits, p65 (RelA) and p50 which are required for activation and nuclear translocation of NF-κB (23). In most quiescent cells NF-κB binds to an inhibitor present in the cytoplasm, I-kappa B (IκB), which inactivates NF-κB by covering the nuclear localization sequence (NLS), blocking DNA binding and nuclear uptake of NF-κB (14).

Extracellular stimuli such as bacteria, virus, cytokines, oncogenic molecules, and chemo/radiotherapy, cell surface receptors including Toll-like receptor (TLR), T/B cell receptor and tumor necrosis factor receptor (TNFR) interact with their specific ligands to cause an up-regulation of the IκB kinase (IKK) complex (24). This complex contains a regulatory subunit IKKγ (NEMO) and catalytic subunits IKKα and IKKβ. IKK complex phosphorylates p65/p50-bound IκB at Serine residues -32 and -36. The phosphorylated IκB is degraded via the ubiquitin-proteasome pathway, allowing for activation of NF-κB. Activated NF-κB has an exposed NLS and is translocated to the nucleus where it binds to enhancer element of the immunoglobulin kappa light-chain of activated B cells (κB sites) triggering down-stream genes expression that potentially promotes inflammation and cancer initiation/progression (24-27).

The alternative NF-κB pathway is initiated by ligands such as cluster of differentiation (CD)-40, B-cell activating factor (BAFF), and lymphotoxin-β receptor (LTBR) and includes RelB/p100 subunits of NF-κB, IKKα homo-dimer, and NF-kB-inducing kinase (NIK) (26). Upon NIK activation, IKKα is phosphorylated and activates RelB by the conversion of p100 to p52 protein. The RelB/p52 complex translocates to the nucleus and leads to the enhanced expression of several genes including BAFF, the stromal cell-derived factor 1 (SDF1), and glycosylation-dependent cell adhesion molecule-1 (GLYCAM1) (28, 29).

Colorectal cancer is a multi-factor disease with various genetic and epigenetic mutations. It has been shown that Mutated Kirsten rat sarcoma viral oncogene homolog (K-RAS) is detected in 30-50% of CRC cases (30). There is a significant correlation between expression levels of NF-κB and abnormal activity of K-RAS in human colorectal adenocarcinoma (P=0.15) (31). In line with this, comparing the active form of NF-κB in tumors with wild type K-RAS and K-RAS mutations showed a higher activity of NF-κB signaling in patients with K-RAS mutations. These patients showed a lower survival and poorer response to first-line treatment, compared to other cases (32, 33). Lin et al. showed that activated NF-κB (P65 subunit) and phosphorylated-IκBα was decreased in colon cancer SW620 cells with KRAS mutations, probably through the RAS/extracellular signal-regulated kinases (ERK)/IκBα signaling pathway (33).

In addition, an adenomatous polyposis coli (*APC*) gene alteration is known as one of the most prevalent events in CRC (34). A study showed that an intestinal stem-cell marker called olfactomedin 4 (OLFM4) negatively suppressed the APC mutation-induced colon carcinogenesis via partial negative regulation of NF-κB pathway. OLFM4 deletion stimulated colon adenocarcinoma in $Apc^{Min/+}$ mice and over-activation of NF-κB was occurred in *Apc Olfm4* double-mutant mice (35). Role of APC gene mutations in the pathogenesis of colorectal cancer has been recently reviewed by Aghabozorgi et al (36).

Role of NF-kB in CRC Cell Proliferation

The NF-κB signaling pathway plays an important role in the regulation of cell proliferation and cell survival. Constitutive activation of this pathway leads to the constitutive expression of proliferation-associated genes including cyclin D1, cyclin E, and cyclin-dependent kinase (CDK)-2, as well as interleukin (IL)-6 and Myc. Since aberrant regulation of NF-κB is frequently reported in tumor cells, inhibition of this cascade may limit cell proliferation (37).

DNA synthesis via thymidylate synthase (TS) and cell cycle progression are necessary for cancer cell proliferation. Rajitha et al. showed that the administration of a potent NF-κB inhibitor, curcumin and its analogs EF31 and UBS109 inhibit the transcription factor E2F-1 and thymidylate synthase (TS) via the suppression of NF-κB activation. Following curcumin treatment, cells were arrested at the G0/G1 boundary, and tumor growth was significantly reduced in the CRC cell lines HCT116 and HT-29 (38). Wang et al. demonstrated that a naphthoquinone compound, lawsone (LS), delays cell cycle progression by down-regulating cyclin B1 and CDK1, interfering with the NF-κB pathway in human colon cancer cell line, DLD-1 and was associated with decreased aberrant crypt and number of adenomas and lesions in CRC (39). Moreover, diterpene lactone obtained from *Andrographis paniculata* and *Jacobinia suberecta*, andrographolide, down-regulates the TLR4/NF-κB/matrix metalloproteinase (MMP)-9 signaling pathway, reducing cell proliferation in the human colon cancer cell line, SW620. Andrographolide also promotes caspase-3/9 activities leading to cell death and enhances cell cytotoxicity (40).

Tetraarsenic hexoxide (As4O6) inactivates TNF-induced NF-κB by inhibiting IκBα phosphorylation, subsequently suppressing proteins involved in proliferation and invasiveness in SW620 cells in *in vitro* and *in vivo* model systems (41). Further studies showed that, hydrogen sulfide (H2S)-releasing naproxen (HS-NAP) which is known as a cardiovascular-safe NSAID, induces G0/G1 arrest, decreasing cell proliferation/survival via NF-κB down-regulation in HT-29 cells. Consistent with these findings, HS-NAP also inhibits tumor volume and tumor progression in a xenograft mouse model (42). It has been shown that diaspirin (DiA) and fumaryl diaspirin (F-DiA), aspirin analogues, significantly inhibit cell proliferation by reducing cyclin D1 levels and promoting the NF-κB pathway, *in vitro* and *in vivo*.

NF-κB regulates CRC cell apoptosis

NF-κB signaling inhibits apoptosis by up-regulating anti-apoptotic genes expression including B-cell lymphoma-extra large (Bcl-xL), the Bcl-2-related gene (A1/BFL1), cellular inhibitors of apoptosis (cIAPs), and caspase-8/FAS-associated death domain-like IL-1beta-converting enzyme inhibitory protein (c-FLIP) (43). Several studies indicate that NF-κB suppression can induce apoptotic cell death in colon cancer cells. Jani et al. have shown that quinacrine abrogates NF-κB activation, and stimulates apoptosis by increasing the cytotoxicity of TNF-related apoptosis-inducing ligand (TRAIL) in human CRC cell lines, RKO and HT29. Two hours exposure of quinacrine down-regulates NF-κB-stimulated anti-apoptotic proteins such as c-FLIP and Mcl-1 sensitizing tumor cells to TRAIL-induced apoptosis. Extended quinacrine treatment for 24 hours decreases the expression of other NF-κB-related survival proteins including survivin, Bcl-2, Bcl-xL, and X-linked inhibitor of apoptosis protein (XIAP) (44).

Zhang et al. have reported that 3,3',4',5,7 pentahydroxyflavone (Quercetin) enhances apoptosis by inactivating the NF-κB signaling pathway, leading to Bcl-2 down-regulation and Bcl-2 Associated X (Bax) up-regulation in stimulated cells (45). Moreover, obovatol, obtained from *Magnolia obovata,* abrogates TNF-α and TPA-induced NF-κB activation by reducing nucleus translocation of p50/p65 and IκB phosphorylation in a dose-dependent manner. Following obovatol administration, the expression of caspase-3, -9, and Bax are increased whereas levels of anti-apoptotic genes including Bcl-2, IAP-1 and XIAP are decreased in SW620 and HCT116 CRC cells (46). Using a saponin extract (CSENS) isolated from *Nigella sativa* decreases NF-κB activity through targeting of the p65 subunit and increasing pro-apoptotic factors such as Bax/Bcl-2 in HCT116 cells. In addition, CSENS enhances chromatin condensation, DNA degradation, cell shrinkage, and cellular detachment (47). The main flavonoid in *Alpinia oxyphylla Miquel*, tectochrysin, activates TRAIL-induced cell death and caspase-3 cleavage through down-regulation of NF-κB signaling and over-expression of death

receptors (DR4, DR3, and Fas) in HT-29 cells. *In vivo* experiments have also shown that high levels of apoptosis reduce tumor volume/weight in a xenograft nude mice (48).

BAY61-3606, a potent inhibitor of the cellular kinase IKKα, sensitizes CRC cells to TRAIL-stimulated apoptosis via suppressing NF-κB and over-expression of DR4 in a P53-dependent manner (49). Similarly, inactivation of NF-κB using an oral butyrate analogue, phenylbutyrate (PB), induces caspase-3-dependent cell death, enhances the mitochondrial membrane potential, and functional Poly ADP-ribose polymerase protein (PARP) (50). To further investigate the anti-apoptotic effects of NF-κB signaling pathway, Kim et al. showed that the hydroxamic acid-derivative, MHY218, inactivates NF-κB and decreases DNA fragmentation, PARP cleavage, caspase activation and alteration in the ratio of Bax/Bcl-2 proteins. Moreover, MHY218 stimulates cell cycle arrest while reducing Cox-2, 5-lipooxygenase, MMP-9, and cyclin B1/Cdc25C/Cdc2 expression (51). Dexamethasone activates apoptosis, increases chemosensitivity and suppresses cell growth via inactivation of NF-κB p65 subunit in colon cancer glucocorticoid receptor α-rich ($GR\alpha^{+}$) cell lines, LoVo and HCT116 (52). These findings support the regulatory role of NF-κB pathway in apoptosis and suggest the clinical value of utilizing specific pharmacological inhibitors of this pathway in preventing CRC progression.

NF-κB inhibitors modulate inflammation in CRC

During development of colitis, bacteria-related lipopolysaccharides (LPS)-induced inflammatory events via NF-κB activation, elevating the levels of inflammatory cytokines (53). Moreover, there is a correlation between NF-κB-induced proliferation and cancer-related inflammation in tumor cells. For instance, Wang et al. showed that GEN-27, a synthetic isoflavonoid, as well as BAY11-7082, an NF-κB inhibitor, suppress IL-1-induced cell proliferation and inflammation by phosphorylating IκB and IKK in HCT116 cells (54). Furthermore, a polyphenol obtained from grapes and red wine, resveratrol, suppresses LPS-induced

inflammation via down-regulation of NF-κB-triggered inducible nitric oxide synthase (iNOS) and nitric oxide (NO) in a dose-dependent manner in human SW480 and Caco-2 cell lines (55).

Fluoxetine, a straight chain phenylpropylamine, attenuates colitis-associated colon cancer by inhibiting TNF-α-induced NF-κB activation and IL-8 expression (56). Another study showed that using 3′-chloro-5,7-dimethoxyisoflavone (CDMF) reduces invasive motility and inflammatory responses in cancer cells. CDMF decreases CXC chemokine ligand 10 (CXCL10)-mediated inflammation by suppressing TNF-α-induced NF-κB activation in HCT116 cells (57). In another study, Nirvanappa et al. showed that 2,2-acetyl-6,6-dimethyl-4-phenyl-5,6-dihydro-2H-1,2-oxazin-3-ylmethyl isoindoline-1,3-dione (API), a synthetic compound, elicits anti-proliferative and anti-inflammatory activities by blocking IκB degradation and NF-κB DNA binding activity in cellular as well as in dextran sulfate sodium (DSS)-induced inflammatory bowel disease (IBD) animal models (58). Moreover, dicaffeoylquinic acids (3,4-diCQA, 3,5-diCQA, and 4,5-diCQA) derived from *Yerba mate* leaves can reduce inflammation by down-regulation of prostaglandin E_2/Cox-2 and NO/iNOS expression through abrogation of NF-κB nuclear translocation reducing cell survival in PKO and HT-29 cancer cells (59).

Targeting NF-κB decreases metastasis in CRC

No significant differences in frequency in the nuclear expression or localization of NF-κB subunits (p50 or p65) were reported for primary and advanced tumors (with liver or lymph node metastasis). These findings suggested that NF-κB activation happen prior to development and metastatic spread and were maintained during progression processes (60). It has been shown that NF-κB signaling pathway was activated by Scaffold attachment factor B (SAFB) reduction during development of colorectal cancer. SAFB prevents the function of transcriptional factors leading transcriptional repression, which its down-regulation modulated the NF-κB activity via targeting transforming growth factor-β–activated kinase 1 (TAK1) involving invasiveness features and poorer patient survival (61). Transcriptional factor NF-κB is involved in cancer-

associated breakdown of extracellular matrix (ECM) and also enhances the expression of various invasiveness-related genes such as MMPs, endothelial leukocyte adhesion molecule 1 (ELAM-1), Vascular cell adhesion molecule 1 (VCAM1), Intercellular Adhesion Molecule 1 (ICAM1), urokinase-type plasminogen activator (uPA), iNOS, and COX2 (37). Moreover, NF-κB signaling including TBK1 affects tumor development via regulation of the Tumor-associated macrophages (TAMs) (M2-like phenotype) in metastatic CRC patients. These results showed that variation in TAM-regulated genes influences clinical outcomes in bevacizumab treated CRC patients (62). Consistent with these findings, Ryan et al. showed that NF-κB suppression prevents invasiveness features of CT-26 colon cancer cells in peritoneal metastasis mice model. High expression levels of IκB-α super-repressor promoted differential polarization of macrophages to anti-oncogenic M1-like phenotype via inhibition of NF-κB. NF-κB-knockdown in cancer cell-conditioned media (CT26/IκB-α SR) over-expresses nitric oxide (NO) synthase and interleukin (IL)-12 in macrophages, and also reduces the expression of matrix metalloproteinase (MMP)-9, and elevates the tissue inhibitor of MMP-1 and -2 (63).

Lin et al. showed that 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside (THSG), extracted from the traditional Chinese herb *Polygonum multiflorum*, reduces invasiveness and migration through decrease in MMP-2 and phosphorylated vascular endothelial (VE)-cadherin followed by IκB phosphorylation in HT-29 cells. THSG also increases transepithelial electrical resistance (TEER) and reduces ICAM-1 and E-selectin proteins decreasing cell adhesion ability in an endothelial cell line, EA.hy926 (64). Furthermore, administration of rapamycin, an immunosuppressant factor inhibiting mTOR signaling, decreases TLR-4, IL-6, and PGE2 levels by deactivating the NF-κB pathway leading to suppression of cancer cell immune escape and invasion in CRC (55). Moreover, a 43-amino acid peptide extracted from soybean, lunasin, interacts with $\alpha_5\beta_1$ integrin and subsequently suppresses migration, attachment, and extravasation partially through inhibition of NF-κB signaling in human CRC cells. Further studies showed that lunasin potentiates the oxaliplatin effects in preventing metastasis *in vivo* and can

be potentially helpful for CRC patient's survival by decreasing metastasis (65). Consistent with these results, 200 μmol/L of genistein, an isoflavone, reverses the epithelial-mesenchymal transition (EMT) and inhibits metastatic phenotype in colon cancer cells. Further studies showed that genistein elicits anti-metastasis effect by E-cadherin up-regulation and N-cadherin down-regulation as well as reduction in EMT markers including forkhead box C1 (FOXC1), FOXC2, zinc finger E-box-binding homeobox 1 (ZEB1), ZEB2, Snail2/slug, and Twist Family BHLH Transcription Factor 1 (TWIST1) followed by suppressing the NF-κB/slug/Notch1/E-cadherin signaling pathways (66). Su et al. have reported that curcumin inhibits the invasion and migration of CRC cells via reduction of NF-κB/p65, Cox-2, and MMP-2 levels while promoting levels of Cox-1 and MMP-9 in COLO205 cells (67).

Another study reported that a Chinese herb isolated from *Sophora flavescens*, oxymatrine regulates the expression of EMT markers (E-cadherin, N-cadherin, and Snail) by inactivation of NF-κB activity in colon cancer cells (68). Similarly, administration of ginsenoside Rg3, derived from Chinese herb ginseng, potently suppresses migration via inactivation of NF-κB transcriptional activity and down-regulation of NF-κB-related genes (MMP-9, Cox-2 and c-Myc) in SW480 colon cancer cells.

Role of NF-kB in resistance to therapy

Resistance to chemotherapy is a critical problem in cancer research and limits the effectiveness of some drugs (69). Several studies showed that activation of NF-κB in response to chemotherapy reduces drug efficacy on tumor death, thus co-administration of chemo agents with NF-κB inhibitors can enhance chemo sensitivity in CRC cells. In line with this, using a NF-κB inhibitor such as bortezomib, SN50 or pyrrolidine dithiocarbamate (PDTC) combined with 5-fluorouracil (5-FU), oxaliplatin, paclitaxel or arsenic acid (As_2O_3) can increase the tumor cell chemosensitivity by inhibiting chemotherapy-stimulated NF-κB activation (70, 71).

Moreover, Wang et al. showed that administration of disulfiram (DS), a drug used as an anti-alcoholism medicine, abrogates the 5-FU-induced NF-κB nuclear translocation and its DNA binding activity, thereby significantly promoting apoptosis and 5-FU cytotoxicity in CRC cell lines, RKO, DLD-1 and H630 (72). Similarly, triptolide, PG490, potentially suppresses 5-FU-induced NF-κB transcriptional activity and enhances the susceptibility of HCT116, PKO and H630 cell lines to 5-FU treatment at least partially by over-expressing caspase-3 and Bax proteins while reducing Bcl-2 expression (73).

Furthermore, a flavonoid extracted from *Epimedii herba* named icariin, also elevates the anti-tumor activities of 5-FU and inhibits tumor growth by suppressing NF-κB activity and its genes products in colorectal cancer cells in both cellular and animal models (74). In another study, curcumin was found to reduce 5-FU-induced IκBα kinase activity, IκBα phosphorylation, NF-κB/Src/PI3K signaling axis, and down-regulates NF-κB target genes. The combined treatment of 5-FU and curcumin potentiates the expression of pro-apoptotic proteins including PARP, Bax, caspase-8, -9 and -3 and reduces anti-apoptotic and proliferative proteins such as Bcl-xL and cyclin D1 in HCT116 cell (75). Porras et al. reported that curcumin also attenuates the resistance of CRC cells to oxaliplatin (OHP) by inactivating the NF-κB signaling pathway and down-regulation of NF-κB-regulated CXC-chemokines including CXCL1, CXCL2 and CXCL8. This combination can affect the outcomes of metastasis in colorectal cancer patients (76). In addition, administration of evodiamine (Evo), an isolated compound from *Evodia rutaecarpa,* attenuates chemotherapy resistance, reduces ATP Binding Cassette Subfamily G Member 2 (ABCG2)-mediated multi-drug resistance while inducing apoptosis by abrogating NF-κB signaling pathway in oxaliplatin-resistant HCT116 cells (HCT-116/L-OHP) (77). The combination of 3-2-bromoethyl indole (BEI-9) with a chemotherapy drug named camptothecin (CPT) elevates the susceptibility of cancer cells to CPT by inactivating NF-κB signaling and modulating expression of its target genes including Cox2 and Bcl-xL in CRC cells. In addition, compared to CPT alone, combined treatment highly increases caspase activity and apoptosis

(78). In line with these results, Lagadec et al. demonstrated that 10μM AS602868, a IKK2 kinase inhibitor, reduced cell growth, and sensitized cancer cells to SN-38, and 5-FU in CRC cells. Moreover, xenograft experiments showed that treatment with AS602868 and CPT-11 increases cell cycle arrest and cell death leading to a reduction of tumor size (79).

Several studies have reported that there is an association between inefficient radiotherapy outcomes and NF-κB activation. Kuo et al. showed that sorafenib, BAY43-9006, decreases radiation-induced NF-κB activity and its co-administration with radiotherapy results in a significant reduction of tumor growth compared to BAY43-9006 or radiation alone (80). Moreover, curcumin reduces radio-resistance by suppressing radiation-stimulated NF-κB activity via inhibition of IKK and IκBα phosphorylation/degradation in human colorectal cancer cells (81). Consistently, icariin, a flavonoid from the herb *Epimedium*, showed similar results and potentiates the radiotherapy effects in a murine model of CRC (82).

A phase II study has confirmed a correlation between activation of NF-κB and resistance to treatment in advanced colorectal cancer cases. Forty-three patients were treated with a combination of FOLFOX-4 regimen and tyrosine kinase inhibitor gefitinib. Median progression-free survival (PFS) and overall survival (OS) were reported 7.8 and 13.9 months, respectively. These results indicated that gefitinib is not able to enhance the effects of FOLFOX, and cannot defeat resistance mechanism due to NF-κB activation (83). These results suggested that inhibition of NF-κB pathway can sensitize colon cancer cells to chemo/radiotherapy and provides more effective strategies to cancer treatment.

Conclusion

There are several studies showing that the NF-κB signaling pathway is activated in various cancer cell lines. Stimulation of NF-κB signaling plays a significant role in the tumorigenesis process via regulation of downstream NF-κB gene products in CRC. Down-regulation of these genes decrease cell proliferation, inflammation, metastasis and

angiogenesis and elevates the levels of apoptotic cell death and drug sensitivity in cancer cells (Figure 1).

Inhibition of NF-κB activity using specific pharmacological inhibitors decreases tumor initiation/development, radiation damage, chemoagent-induced adverse effects, and acute inflammatory responses. Thus, using a pharmacological inhibitor of NF-κB as an adjuvant treatment with chemo/radiotherapy enhances the synergistic effects and is considered as a novel approach for therapeutic strategies.

However, the role of NF-κB signaling in CRC is complex. The efficacy of these NF-κB inhibitors should be investigated in patients with CRC and further investigation should be performed in this regard to determine the molecular mechanism and the exact role of each inhibitor in tumorigenic responses. The information gained from all these studies helps in the design of novel selective NF-κB inhibitors and has great clinical significance in the CRC treatment strategies.

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Figure legend

Figure 1. Regulatory roles of NFkB signaling pathway in the pathogenesis of CRC.